Sensitivity to theta-burst timing permits LTP in dorsal striatal adult brain slice

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Submitted 14 February 2013; accepted in final form 2 August 2013

Hawes SL, Gillani F, Evans RC, Benkert EA, Blackwell KT. Sensitivity to theta-burst timing permits LTP in dorsal striatal adult brain slice. J Neurophysiol 110: 2027–2036, 2013. First published August 7, 2013; doi:10.1152/jn.00115.2013.—Long-term potentiation (LTP) of excitatory afferents to the dorsal striatum likely occurs with learning to encode new skills and habits, yet corticostratial LTP is challenging to evoke reliably in brain slice under physiological conditions. Here we test the hypothesis that stimulating striatal afferents with theta-burst timing, similar to recently reported in vivo temporal patterns corresponding to learning, evokes LTP. Recording from adult mouse brain slice extracellularly in 1 mM Mg2+, we find LTP in dorsomedial and dorsolateral striatum is preferentially evoked by certain theta-burst patterns. In particular, we demonstrate that greater LTP is produced using moderate intraburst and high theta-range frequencies, and that pauses separating bursts of stimuli are critical for LTP induction. By altering temporal pattern alone, we illustrate the importance of burst-patterning for LTP induction and demonstrate that corticostriatal long-term depression is evoked in the same preparation. In accord with prior studies, LTP is greatest in dorsomedial striatum and relies on N-methyl-D-aspartate receptors. We also demonstrate a requirement for both Gαs and Gαout-coupled pathways, as well as several kinases associated with memory storage: PKC, PKA, and ERK. Our data build on previous reports of activity-directed plasticity by identifying effective values for distinct temporal parameters in variants of theta-burst LTP induction paradigms. We conclude that those variants which best match reports of striatal activity during learning behavior are most successful in evoking dorsal striatal LTP in adult brain slice without altering artificial cerebrospinal fluid. Future application of this approach will enable diverse investigations of plasticity serving striatal-based learning.

LTP; striatum; theta; plasticity; learning

THE DORSAL STRIATUM HOSTS an intersection of cognitive, limbic, motor, and reward systems which combine to impart neural changes underlying learning. Striatal activity is critical for instrumental learning (Graybiel 1995; Yin et al. 2005), motor skill development (Yin et al. 2009), cued action-selection (Packard and Teather 1997), and habit formation (Yin and Knowlton 2006). Distinct types and stages of learning differ in their engagement of medial and lateral dorsal striatal regions (Pauli et al. 2012; Yin et al. 2006). Observations in vivo and ex vivo suggest that changes in strength of connection between neurons underlie striatal learning and memory (Koralek et al. 2012; Pascoli et al. 2011; Pauli et al. 2012; Shen et al. 2011; Yin et al. 2009). Stimulation of glutamatergic and dopaminergic afferents to dorsal striatum leads to in vivo corticostriatal long-term potentiation (LTP), the strength of which correlates with learning speed (Chargier et al. 1999; Reynolds et al. 2001). Rotarod training reduces long-term depression (LTD) ex vivo in the dorsal striatum (Yin et al. 2009). Despite its importance in learning and memory, investigation of mecha-

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The success of TBS to induced LTP in the hippocampus, together with the emergence of theta-rhythms in dorsal striatum during learning, suggests that similar protocols may induce physiologically realistic LTP in ex vivo striatum. In this study, we test the hypothesis that learning-related temporal patterns, in the form of TBS, will induce long-lasting striatal LTP. We find that delivering stimuli in physiological bursts at a behaviorally relevant theta-range frequency induces late-phase striatal LTP in adult tissue without altering ionic composition, and we identify molecular effectors serving striatal theta-burst LTP.

MATERIALS AND METHODS

All animal handling and procedures were in accordance with the National Institutes of Health animal welfare guidelines and were approved by the George Mason University IACUC. Male C57BL/6 mice (2–5 mo) were decapitated while anesthetized using isoflurane. Brains were extracted into ice-cold, oxygenated slicing solution (in mM: NaCl 126, NaH2PO4 1.25, KCl 2.8, dextrose 10, NaHCO3 26.2, CaCl2 1.25, MgCl2 0.5, MgSO4 7, sucrose 210) and coronally sectioned at 350 μm using a vibratome (Leica VT 1000S). Slices were collected anterior to, and including the level of, the anterior commissure. Slices were bisected by hemisphere and placed in an incubation chamber containing aCSF including the level of, the anterior commissure. Slices were evoked by stimulating white matter overlaying striatum with a tungsten bipolar electrode at an intensity producing 40 – 60% of the peak signal amplitude on an input-output curve. In most recordings, we identify molecular effectors serving striatal theta-burst LTP.

In pharmacology experiments, TBS was delivered to one hemislice, and the other served as a nonstimulated control for nonspecific drug effects on signal size. Drugs were bath applied at least 20 min prior to induction and maintained throughout experiments. Salts were purchased from Fisher Scientific. Picrotoxin, cherythrine chloride, P(KH14-22) amide, telenzepine dihydrochloride, and (R5)-1-aminoo- dan-1,5-dicarboxylic acid (AIDA) were purchased from Tocris Bioscience, and both 2-amin-5-phosphonovaleric acid (APV) and SCH23390 were purchased from Enzo Life Science. All drugs were water soluble. Picrotoxin was weighed as a powder and added to aCSF daily as needed, while all other drugs were dissolved in water and stored in concentrated aliquots to be added to aCSF as needed.

RESULTS

Corticostriatal LTP is improved by approximating physiological frequencies. Using field recordings in the dorsal striatum, we examined the efficacy of TBS paradigms to induce corticostriatal LTP in dorsomedial (DM) and dorsolateral (DL) striatum in adult mouse brain slice bathed in aCSF containing physiologigical Mg2+ (1 mM). GABA_A activity was consistently blocked to isolate the striatal response to glutamatergic synapses. Distinct protocols to be compared were administered to neighboring coronal hemislices in a common chamber. Two temporal features of the induction pattern were varied: intraburst frequency and theta-burst frequency (Fig. 1A).

Intraburst frequencies of 50 and 100 Hz were compared while maintaining 10.5-Hz theta frequency. Our results indicate that 50-Hz intraburst produces stronger LTP than 100 Hz in both DM and DL regions (Fig. 1B). Both 50 and 100 Hz produced a significant LTP compared with nonstimulated controls. Statistical analysis of the results (Table 1) using repeated-measures GLM shows that 50-Hz LTP was significantly better than 100 Hz, with 50 Hz producing larger and longer lasting LTP than 100 Hz, and DM striatum supporting stronger potentiation than DL [at 60 min: intraburst F(2, 57) = 11.55, P < 0.0001; region F(1, 57) = 10.07, P = 0.003]. The more pronounced 50-Hz LTP was recorded out to 120 min (see Fig. 1C), by which time DM striatum was potentiated 129 ± 5% and differed significantly from nonstimulated controls (93%), while DL striatum, at 106 ± 4%, did not (data not shown) at 120 min: GLM on intraburst F(2, 37) = 21.37, P < 0.0001; LSmens vs. nonstimulated controls: DM P < 0.0001, DL P =
Since DM LTP was stronger than DL LTP, we subsequently focused on the DM striatum, using the more effective 50-Hz intraburst frequency.

Working in the DM striatum, we tested the effect of three different frequencies spanning the theta range (5–11 Hz): 5 Hz, 8 Hz, and 10.5 Hz. Repeated-measures GLM shows significant effects of theta frequency \([F(3,46) = 10, \ P < 0.0001\) at 120 min\] and time \([F(3,96) = 23.25, \ P < 0.0001\] with higher theta frequencies inducing the greatest and longest lasting potentiation (Fig. 1C). Post hoc analysis indicates that the 10.5-Hz group, which produced late-phase LTP by maintaining 129 ± 5% potentiation 120 min postinduction, differs significantly from nonstimulated controls (LSmeans vs. controls, \(P < 0.0001\)). The same analysis reveals that LTP evoked by 8-Hz theta is not well maintained, losing significance by 120 min (at 60 min: 128 ± 10%; \(P = 0.001\); at 120 min: 112 ± 9%, \(P = 0.05\)), and that the small LTP evoked by 5-Hz theta (at 60 min: 116 ± 5%, \(P = 0.04\) has dissipated by 120 min (108 ± 4%, \(P = 0.13\)). These differences in LTP strength cannot be attributed to different initial amplitude, as average baseline population spike amplitude did not differ among the four DM theta-burst paradigms and nonstimulated controls [GLM, \(F(4,77) = 1.49, \ P = 0.21\)]. In summary, the optimal TBS (50 Hz intraburst; 10.5 Hz theta) is the only protocol that produces a long-lasting LTP and thus is used for the remainder of our investigations.

**Burstiness is critical to striatal TBS LTP.** We find that lower intraburst and higher theta frequencies are more effective for LTP induction; however, as theta frequency increases, the...
pause separating bursts is reduced. We therefore tested the importance of burst-patterning by eliminating the theta component of our induction protocol by decreasing the interburst pause from 35 ms (using the optimal 10.5-Hz theta) to 20 ms. In other words, we delivered trains of stimuli at an unbroken 50 Hz in a “nonbursty” induction variant in which train number, intertrain interval, and the number of stimuli delivered remained matched to TBS protocols (Fig. 1A). Despite close temporal similarity to the optimal TBS, the nonbursty stimulation failed to evoke LTP (Fig. 1C, Table 1). Statistical analysis implicates burstiness as a significant factor contributing to LTP induction [repeated-measures GLM, F(2,34) = 13.89, P < 0.0001]. Post hoc analysis indicates significant difference between TBS and nonbursty groups (LSmeans, P < 0.05) and no difference between nonbursty stimulation and nonstimulated controls (LSmeans, P > 0.05). The 35-ms pause between bursts when using the optimal 10.5-Hz theta frequency provides a mere 15-ms increase relative to the 20-ms break dividing 50-Hz stimuli within nonbursty trains. Our data identify this brief pause as a critical feature enabling long-lasting TBS LTP.

**TBS LTP is present, although less consistent, when GABA_A inputs remain active.** To isolate the contribution of glutamatergic synapses onto medium spiny neurons, TBS optimization was carried out in 50 μM picrotoxin, eliminating GABAAergic interneuron and medium spiny collateral influence. Thus, to assess the effect of GABAAergic inputs on TBS-induced synaptic plasticity, the optimal TBS was administered to the DM striatum as before, but picrotoxin was omitted from the aCSF. In the absence of picrotoxin, the net effect of TBS remains LTP (Fig. 1D, Table 1). On average, population response following TBS in the absence of picrotoxin remained larger than nonstimulated controls [GLM, F(1,18) = 4.59, TBS without picrotoxin at 60 min: 114 ± 9% vs. controls 95 ± 3%, P = 0.02; TBS without picrotoxin at 120 min: 110 ± 7% vs. controls 93 ± 4%, P = 0.01]. However, isolation of glutamatergic influence on medium spiny neurons using picrotoxin improves consistency in TBS-evoked LTP; therefore, picrotoxin was used in all subsequent investigations.

**Bidirectional plasticity is obtained through temporal pattern.** We tested the ability of our preparation to express bidirectional plasticity to validate the utility of our theta-burst paradigm for evaluating how temporal pattern influences plasticity. First, we applied HFS (see Fig. 1A) commonly used to induce corticostriatal LTD in the presence of Mg^{2+} (Lerner and Kreitzer 2011), although in some instances it evokes LTP (Fino et al. 2005) or variable plasticity (Akopian et al. 2000; Akopian and Walsh 2006; Spencer and Murphy 2000). Applying the HFS protocol, we induced a small, transient increase in signal size dorsomedially (Fig. 2, Table 1) and induced no plasticity dorsolaterally (Fig. 2, Table 1). A variant of this protocol in which stimulation intensity during HFS is increased produced a similar result (data not shown). In summary, HFS did not produce a significant difference from nonstimulated controls at 30 min [GLM, F(1,29) = 0.01, P = 0.93].

Next we evaluated a more moderate frequency induction paradigm, as this has shown success in promoting striatal LTD (Lerner and Kreitzer 2012; Ronesi and Lovinger 2004). In both striatal regions, we delivered pulses in the same four-train structure as HFS, but employed a moderate 20-Hz frequency within trains (see Fig. 1A). Four trains of 20 Hz evoked LTD, with DL showing greater LTD than DM striatum [Fig. 2; repeated-measures GLM, F(2,49) = 16.46, region P = 0.03, stimulation P < 0.0001]. The ability of the 20-Hz stimulation to evoke LTD dorsomedially demonstrates the capacity of our adult tissue preparation to reliably display LTD, as well as LTP, through manipulation of temporal pattern alone.

**Theta-burst LTP requires NMDA and G_q- and G_s/olf-coupled receptors.** Collaborative signaling by neurotransmitters glutamate, acetylcholine, and dopamine is critical to striatal learning and plasticity (Lerner and Kreitzer 2011). Glutamate at active NMDA receptors provides calcium influx supporting learning and LTP. Metabotropic glutamate receptors (mGluR) on medium spiny neurons have demonstrated involvement in LTD using 0 Mg^{2+} HFS (Gubellini et al. 2003). Theta-burst may optimize acetylcholine release (Zhang et al. 2010), potentially activating G_q-coupled signaling pathways in common with mGluR (Calabresi et al. 1998, 1999). Dopamine acting at

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**Table 1. Plasticity by region and induction variant**

<table>
<thead>
<tr>
<th>Region</th>
<th>Intraburst, Hz</th>
<th>Theta, Hz</th>
<th>Four Train, Hz</th>
<th>%Baseline 60 min</th>
<th>%Baseline 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM/L†</td>
<td>50</td>
<td>10.5</td>
<td></td>
<td>95 ± 3</td>
<td>93 ± 4</td>
</tr>
<tr>
<td>DM</td>
<td>100</td>
<td>10.5</td>
<td></td>
<td>119 ± 4</td>
<td></td>
</tr>
<tr>
<td>DL</td>
<td>50</td>
<td>10.5</td>
<td></td>
<td>110 ± 4</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>DL</td>
<td>100</td>
<td>10.5</td>
<td></td>
<td>95 ± 4</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>50</td>
<td>8</td>
<td></td>
<td>128 ± 10</td>
<td>112 ± 9</td>
</tr>
<tr>
<td>DM</td>
<td>50</td>
<td>5</td>
<td></td>
<td>116 ± 5</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>DM</td>
<td>50‡</td>
<td>10.5‡</td>
<td></td>
<td>114 ± 9</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>DM</td>
<td>50*</td>
<td>12.5*</td>
<td></td>
<td>97 ± 7</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>100</td>
<td>109 ± 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>20</td>
<td>80 ± 7</td>
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</tr>
<tr>
<td>DL</td>
<td>20</td>
<td>68 ± 5</td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SE. DM, dorsomedial; DL, dorsolateral. †Nonstimulated controls. These did not differ regionally; thus DM and DL controls were pooled for analysis. ‡No picrotoxin was used. *The nonbursty induction variant.
Gsolf-coupled D1-type (D1 and D5) dopamine receptors is critical to LTP in both populations of medium spiny neurons (Kerr and Wickens 2001; Pawlak and Kerr 2008). We bath applied antagonists specific to these receptors to evaluate their role in TBS LTP. Simultaneous recordings from paired hemislices, one nonstimulated and one TBS stimulated, controlled for nonspecific drug effects. A contemporaneously interleaved cohort of drug-free TBS (50 Hz intraburst, 10.5 Hz theta) is used for comparison.

Figure 3 illustrates the effects of receptor antagonists on TBS-induced plasticity. The NMDA-type glutamate receptor antagonist APV (50 μM) fully prevents TBS LTP [Fig. 3A, Table 2; GLM, F(2,35) = 28.93, P < 0.0001], confirming a requirement for NMDA receptor activation. Next, we independently block m1 type metabotropic acetylcholine (mAChR) and group I glutamate (mGluR1/5) receptors. Both the m1 AChR antagonist AIDA (100 μM) and mGluR1/5 antagonist telenzepine (300 nM) individually abolish TBS LTP without affecting unstimulated control slices [Fig. 3B and C, Table 2; AIDA: GLM, F(2,41) = 14.04, P < 0.0001; telenzepine: GLM, F(2,33) = 48.85, P < 0.0001]. This suggests that Gq activation is needed through both glutamate and acetylcholine, as neither is sufficient to support TBS LTP alone. Bath application of an antagonist selective for Gsolf-coupled dopamine receptors, SCH23390 (10 μM), abolishes TBS LTP by 30 min without affecting unstimulated control slices [Fig. 3D, Table 2; SCH23390: GLM, F(2,36) = 26.02, P < 0.0001], confirming

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Inhibits</th>
<th>%Baseline 30 min, TBS</th>
<th>%Baseline 30 min, No Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>APV</td>
<td>NMDA receptor</td>
<td>91 ± 2</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>AIDA</td>
<td>Group I mGluR</td>
<td>95 ± 10</td>
<td>96.3 ± 6</td>
</tr>
<tr>
<td>Telenzepine</td>
<td>m1 receptor</td>
<td>85 ± 12</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>SCH23990</td>
<td>D1/5 receptor</td>
<td>97 ± 6</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>CHE</td>
<td>PKC</td>
<td>92 ± 10</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>PGI</td>
<td>PKA</td>
<td>105 ± 8</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>CHE+PKI</td>
<td>PKC and PKA</td>
<td>86 ± 15</td>
<td>93 ± 7</td>
</tr>
<tr>
<td>U-0126</td>
<td>ERK</td>
<td>97 ± 9</td>
<td>101 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. See text for definition of acronyms.
a requirement for dopamine activation of $\mathrm{G_{olf}}$-coupled pathways. Post hoc analysis for each of the above antagonists shows no difference in population spike amplitude over time between nonstimulated and TBS-treated slices in the presence of drug (LSmeans, $P > 0.9$). These results confirm that TBS LTP shares receptor dependence with striatal learning and established plasticity.

**Theta-burst LTP requires PKC, PKA, and ERK.** Next we tested several kinases downstream of these implicated receptors to identify further effectors serving TBS LTP. The combination of NMDA-derived calcium and $\mathrm{G_{olf}}$-signaling creates the potential for activating protein kinase $\mathrm{C}$ (PKC), a kinase which may serve striatal LTP (Calabresi et al. 1998; Gubellini et al. 2004). $\mathrm{G_{olf}}$-signaling elevates cAMP and activates protein kinase $\mathrm{A}$ (PKA), a second kinase with a likely role in striatal LTP (Spencer and Murphy 2002). Extracellular signal-regulated kinase (ERK) is a kinase activated downstream of either PKC or PKA and has important roles in memory, drug addiction, and long-lasting plasticity (Mazzucchelli et al. 2002; Shiflett and Balleine 2011). We bath applied antagonists to these kinases during TBS experiments, again recording from paired hemislices, one nonstimulated and one TBS stimulated, to control for nonspecific drug effects.

Figure 4 illustrates the effects of kinase antagonists on TBS-induced plasticity. Bath-applied PKC antagonist, chelerythrine (6–10 $\mu\text{M}$), significantly reduces TBS LTP without affecting unstimulated control slices [Fig. 4A; Table 2; GLM, $F(2,33) = 37.27$, $P < 0.0001$]. Similarly, bath-applied cell-permeant PKA inhibitor peptide, PKI (1 $\mu\text{M}$), significantly reduces TBS LTP without affecting unstimulated control slices [Fig. 4B; Table 2; GLM, $F(2,39) = 25.28$, $P < 0.0001$]. Since PKI did not completely block LTP, we evaluated its effect in combination with chelerythrine. We used reduced concentrations of both antagonists, each showing reduced efficacy to block LTP (Fig. 4; reduced chelerythrine at 30 min: 114 $\pm$ 10; reduced PKI at 30 min: 117 $\pm$ 10). This reduced concentration combination fully prevents TBS LTP [Fig. 4C; Table 2; GLM, $F(2,33) = 15.31$, $P < 0.0001$], demonstrating that PKC and PKA cooperatively support striatal LTP. Bath-applied MAPK/ERK kinase (MEK) inhibitor U-0126 (30 $\mu\text{M}$) prevents MEK...
from activating ERK and fully blocks TBS LTP (Fig. 4D, Table 2; GLM, \(F(2,32) = 18.4, P < 0.0001\)); this effect is similar to the combination of antagonists to PKA and PKC, either of which can act upstream of ERK. Our results newly implicate PKC in activity-dependent striatal LTP and agree with prior studies implicating PKA and ERK (Calabresi et al. 1992b; Kerr and Wickens 2001). These results further suggest that PKA and PKC cooperatively serve LTP, which could occur through coactivation of ERK.

DISCUSSION

Learning correlates with theta frequency neural activity in the dorsal striatum, suggesting a theta-burst induction paradigm might evoke behaviorally relevant LTP in striatum. Our results support this hypothesis by showing that TBS evokes LTP which is more pronounced when using temporal parameters with better correspondence to striatal physiology. We further determine that a critical induction feature for LTP is burstiness, which is intriguing since medium spiny neuron up-state potentials observed in organotypic culture and in vivo may facilitate the burst firing that has demonstrated importance for striatal network function and behavior (Kerr and Plenz 2002; Miller et al. 2008; Stern et al. 1997). Importantly, reliance on in vivo striatal theta rhythms rather than altered ionic composition or pharmacology makes TBS LTP a convincing ex vivo model for plasticity, serving learning, memory, and motor adaptation. Indeed, we confirm involvement of several receptors and kinases previously implicated in striatal plasticity, as well as learning and memory. The success of TBS LTP across dorsal striatal regions in adult brain slice presages its utility in combination with future behavioral studies.

Although both 50-Hz and 100-Hz stimulation frequencies have been applied to evoke plasticity, the striatal medium spiny neurons comprising 95% of striatal cells are not likely to be engaged by high-frequency activation in vivo. These neurons receive input from layer V cortical neurons, which fire with an average rate of 5–10 Hz (Fellous et al. 2003; Wilson and Groves 1981). Furthermore, recordings from behaving mice and rats have shown medium spiny neurons fire below 5 Hz on average, with the maximum spontaneous firing rate in vivo no greater than 50 Hz (Barnes et al. 2005; Miller et al. 2008). In anesthetized rat, single striatal neurons are successfully entrained to moderate (20 Hz), but not to high-frequency (100 Hz) cortical afferent stimulation (Schulz et al. 2011). Given these observations in the literature, we expected and indeed obtained the greatest plasticity through use of more moderate induction frequencies.

Theta frequency is a physiologically significant parameter in activity-based LTP induction as dorsal striatal local field potentials recorded in vivo demonstrate neuronal population coherence at theta-range frequencies (5–11 Hz). Importantly, these theta rhythms are modulated in an activity-dependent manner during learning (Buzsáki 2005; Koralek et al. 2012; Tort et al. 2008). Depolarizing potentials in medium spiny neurons occur at 5 Hz as a result of 5 Hz coherence in firing among hundreds of convergent afferents from layer V cortex in anesthetized rat (Charpier et al. 1999). Higher theta-range frequencies may dominate in wakeful animals, or during learning, since recent studies in awake, behaving subjects indicate that learning-related theta centers around 7–11 Hz in dorsal striatum (DeCoteau et al. 2007; Tort et al. 2008). Nonetheless, we initially used 5-Hz because 5-Hz TBS evokes robust LTP in hippocampal slice (Larson et al. 1986; Nie et al. 2007), and striatal STDP pairings paced at 5 Hz evoke LTP in young animals (Shen et al. 2008). While 5 Hz indeed evoked a modest LTP, we found the amplitude and duration were greatly improved by using higher frequency theta-bursts. This result suggests that LTP processes may be tuned to subtly different frequencies in striatum vs. hippocampus. In light of the recent in vivo work mentioned, this result supports the idea that striatal neurons are tuned to promote LTP in response to temporal patterns emerging with learning behavior.

In optimizing a theta-burst protocol, we eliminated fast actions of intrastratal GABA release which are present in vivo to provide certainty that TBS potentiates the response of medium spiny neurons to glutamatergic afferents rather than depressing the fast GABAergic inhibition of striatal response. This is a valid concern because GABAergic synapses within striatum are more sensitive to endocannabinoid-dependent depression than are glutamatergic synapses (Ademark and Lovinger 2009). Although most corticostratal plasticity studies are carried out with GABA\(_A\) blocked (Akopian and Walsh 2006; Gubellini et al. 2003; Kerr and Wickens 2001; Shen et al. 2008), native GABA\(_A\) transmission must shape striatal plasticity.

Indeed, the direction of STDP is reversed by the presence of GABA\(_A\) antagonists. Specifically, corticostratal synapses onto medium spiny neurons are potentiated when presynaptic release precedes postsynaptic depolarization (Hebbian LTP) only when GABA\(_A\) is blocked; when GABA\(_A\) is not blocked, pre-post pairing is depressing, and LTP is instead evoked when postsynaptic depolarization precedes presynaptic release (anti-Hebbian) (Fino et al. 2010). Several mechanisms have been proposed to account for reversal of STDP by GABA\(_A\), such as altered ratio of NMDA to L-type calcium influx in dendrites (Paille et al. 2013) or Hebbian potentiation of feed-forward inhibition (Fino et al. 2008). Alternatively, increased dopamine release may be responsible for switching STDP direction (Shen et al. 2008; Shindou et al. 2011), since GABA\(_A\) antagonists increase activity-dependent intrastratal dopamine release (Juranyi et al. 2003). Any of these mechanisms, altered calcium source, potentiation of feed-forward inhibition, or lowered dopamine release, may contribute to reduce TBS LTP amplitude in the absence of picrotoxin.

The optimal TBS protocol induces robust DM LTP lasting multiple hours, yet no plasticity results if the brief pause separating bursts is omitted. This demonstrates that the 35-ms pause between bursts is critical to LTP, since no plasticity is induced if this pause is reduced to 20 ms (so that pulses run together into nonbursty, 50-Hz stimuli). Note that TBS variations with lower intraburst or higher interburst frequencies cannot be tested while conserving pulse number per burst, as these adjustments would encroach on the already small interburst pause, eliminating burstiness. The requisite pause may enable LTP through phasic activation of neuromodulators, given that salient behavioral stimuli produce burst firing of cholinergic interneurons (Aosaki et al. 1994) which in turn enhances dopamine release (Threlfell et al. 2012). This may be tested using voltammetry to compare dopamine release resulting from TBS and its nonbursty counterpart. Alternatively, the pause may enable desensitization of critical plasticity effectors.
For instance, a brief break in stimulation may relieve inactivation of NMDA receptors, or else it might relieve desensitization of mGluR, dopamine receptors, or AChR implicated in this study. Investigating the requisite pause may shed light on a mechanism for the resilience of TBS LTP in the absence of GABA_A antagonist.

Striatal sensitivity to temporal pattern is most meaningful if both LTP and LTD can be evoked in the same preparation; therefore, we sought to induce LTD by varying temporal pattern alone. HFS is commonly used to evoke LTD in Mg^{2+}-containing aCSF (Adersmark and Lovinger 2009; Choi and Lovinger 1997; Wang et al. 2006; Yin et al. 2009), yet four-train, 100-Hz HFS does not evoke lasting plasticity in our preparation. This may be related to animal age, which is known to influence evoked striatal plasticity (Partridge et al. 2000), and at least one report notes that 100-Hz HFS does not reliably produce LTD in adult animals (Hopf et al. 2010), while other studies report a mixture of LTD and LTD as a result of HFS in adults (Akopian et al. 2000; Akopian and Walsh 2006; Spencer and Murphy 2000). Our preparation demonstrates reliable LTD across regions when stimulation is delivered at a moderate, 20-Hz frequency, similar to protocols used previously (Lerner and Kreitzer 2012; Yin and Lovinger 2006). In addition to being more effective, 20 Hz is more physiological than 100 Hz, given the moderate native firing frequencies in cortical afferents and striatal medium spiny neurons (Schulz et al. 2011). Capacity for bidirectional plasticity in both DM and DL striatum through manipulation of stimuli timing alone argues against our preparation being skewed toward generating LTP. This strengthens our findings that temporal features of TBS, such as frequency-tuning and burstiness, can modulate LTP strength.

In addition to confirming a requirement for NMDA receptors, our experiments demonstrate that TBS LTP requires G_Gq-coupled metabotropic receptors responding to glutamate and AChR, and G_Gs/olf-coupled dopamine receptors. G_Gq effectors interact with calcium influx to generate 2-arachidonyl glycerol, an endocannabinoid implicated in LTD, and also lead to PKC activation. PKC has been implicated in plasticity, memory, and in striatal chemical LTD (Diez-Guerra 2010; Gubellini et al. 2004) and is a critical intermediary for neuro-modulation of NMDA and AMPA receptors within striatum (Ahn and Choe 2010; Calabresi et al. 1998). We find that independently blocking group I mGluR, m1 AChR receptors, or PKC is sufficient to fully prevent TBS LTD, suggesting that coordinated glutamate and acetylcholine transmission is needed to generate LTD-supportive PKC. The neurotransmitter dopamine acts at G_Gs/olf-coupled D1-type receptors (D1 and D5) expressed on several cell classes within striatum, including both classes of medium spiny neuron (Riviera et al. 2002; Surmeier et al. 1996). D1-type dopamine receptors (along with A2A adenosine receptors) are G_Gs/olf-coupled, leading to elevations in cyclic AMP and PKA. PKA has demonstrated a role in learning and is believed to serve striatal LTP by enhancing medium spiny neuron responsiveness (Dudman et al. 2003; Tseng et al. 2007). Our finding that D1-type dopamine receptor antagonist blocks LTD is consistent with studies of striatal LTD induced using either four-train HFS in 0 Mg^{2+} or STDP (Calabresi et al. 1992a; Fino et al. 2010; Kerr and Wickens 2001; Pawlak and Kerr 2008; Shen et al. 2008). Indeed, D1-type receptor antagonist blocks 0 Mg^{2+} LTP equally well in all patched medium spiny neurons, with the same time course we show (Calabresi et al. 2000; Kerr and Wickens 2001). Thus D1/D5 receptor activity likely increases PKA within medium spiny neurons, which we demonstrate contributes to LTD. Activation of PKA has been demonstrated to accelerate degradation of G_Gq proteins needed to generate endocannabinoids and active PKC (Lerner and Kreitzer 2012); thus the requirement for two sources of G_Gq may stem from the need to overcome PKA obstructing PKC activation. Together our results demonstrate that neither G_Gq nor G_Gs/olf signaling is independently sufficient to support TBS LTD, and that both contribute.

Persistent memory, late-phase plasticity, and long-lasting TBS LTD are each reliant on the kinase and transcriptional regulator ERK (Adams et al. 2000; Valjent et al. 2001). ERK is important for striatal learning, especially that associated with drug addiction (Shiflett and Balleine 2011; Valjent et al. 2006). It is noteworthy that both kinases PKC and PKA are capable of raising ERK phosphorylation and activity (Mao et al. 2005; Shiflett and Balleine 2011). Thus the cooperativity we see when combining low concentrations of PKC and PKA antagonists may result from concomitant reduction in these two sources of ERK phosphorylation. Two possibilities can be distinguished in future works by measuring the effect of PKA and PKC inhibitors on TBS LTD from identified D1 and D2 medium spiny neurons: PKC and PKA may act together upstream of ERK in each medium spiny neuron; or else PKC and PKA may be differentially critical to ERK activation between medium spiny neuron classes. Identifying roles for effectors known to be critical to learning and long-term memory storage strengthens TBS as a model for behaviorally relevant plasticity.

We find DM striatum more prone to potentiation and DL more prone to depression, agreeing with numerous reports (Partridge et al. 2000; Smith et al. 2001; Wickens et al. 2007). Striatal regional gradients exist for several plasticity effectors. For instance, LTD-required endocannabinoid receptors are denser laterally (Hilario et al. 2007). An established medial-to-lateral gradient in NMDA receptor subunit composition and distribution (Chapman et al. 2003; Yin et al. 2009) may cause regional differences in calcium-dependent plasticity effectors, including endocannabinoids and PKC. Dorsolaterally, greater dopamine innervation paired with higher density G_Gs/olf-coupled D2-type dopamine receptors (Doucet et al. 1986; Yin et al. 2009) could limit LTD-supportive PKA in this region. The trend toward greater LTD in DM relative to DL striatum is maintained whether or not GABA_A is blocked (Smith et al. 2001); and our use of picrotoxin rules out regional differences in plasticity of GABA_A transmission. Whatever the reasons for greater LTD magnitude and duration dorsomedially, the current utility of TBS LTD in either region (albeit reduced laterally) will be valuable for investigating learning, since dorsal striatal regions serve distinct styles and phases of learning (Pauli et al. 2012; Yin et al. 2006).

Importantly, dorsal striatum expresses long-lasting potentiation in response to physiological activity patterns similar to those occurring with learning, namely TBS. The extensive duration of DM TBS LTD will accommodate evaluation of late-phase LTD in this region. Utility in adult tissue will benefit behavioral approaches to striatal research, since working with adult animals avoids developmental confounds. Moreover, we have success with TBS LTD in tissue from adult Long Evans rat, a more versatile model for behavior than mice (Hawes et al.
2012). Thus TBS improves on existing LTP protocols in its capacity to merge plasticity with behavioral studies, generating exciting opportunity for advancing knowledge of striatal neurobiology serving learning and memory.

GRANTS

Support from Office of Naval Research Multidisciplinary University Research Initiative Grant N00014-10-1-0198 and NIAAA R01 is gratefully acknowledged.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Authors: S.L.H. and K.T.B. conception and design of research; S.L.H., F.G., R.C.E., and E.A.B. performed experiments; S.L.H., F.G., and K.T.B. analyzed data; S.L.H. and K.T.B. interpreted results of experiments; S.L.H. prepared figures; S.L.H. drafted manuscript; S.L.H. and K.T.B. edited and revised manuscript; S.L.H., F.G., R.C.E., and K.T.B. approved final version of manuscript.

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J Neurophysiol • doi:10.1152/jn.00115.2013 • www.jn.org

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